

## PAPER

## The promise of gene therapy in gastrointestinal and liver diseases

J Prieto, M Herraiz, B Sangro, C Qian, G Mazzolini, I Melero, J Ruiz

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Gene therapy consists of the transfer of genetic material to cells to achieve a therapeutic goal. In the field of gastroenterology and hepatology gene therapy has produced considerable expectation as a potential tool in the management of conditions that lack effective therapy including non-resectable neoplasms of the liver, pancreas and gastrointestinal tract, chronic viral hepatitis unresponsive to interferon therapy, liver cirrhosis, and inflammatory bowel disease.

## BASIC CONCEPTS

Gene therapy is a new approach to treat human diseases based on the transfer of genetic material to the cells. The transferred genetic material is most commonly a natural gene but it can also be a chimeric gene or subgenomic molecule. A cell is said to be transduced when it has incorporated and expresses a foreign gene.<sup>1</sup> To facilitate cell transduction, the genetic material is packaged into molecular constructs named vectors, which can be of viral<sup>2</sup> and non-viral nature.<sup>3</sup> Viral vectors are frequently preferred because of their higher transduction efficiency and can be classified as long term and short-term expression vectors (retroviruses, adeno-associated viruses (AAV), gutless adenoviruses belong to the first category and first generation adenoviruses to the second).

For a gene to be expressed inside a cell its coding DNA sequence should be linked to appropriate regulatory DNA sequences. Gene promoters (and other regulatory elements) may allow transgene expression in every transduced cell (universal promoters)<sup>4</sup> or, alternatively, only in selected cells containing transcription factors able to interact specifically with the promoter (as in the case of tumour specific promoters).<sup>5</sup> On the other hand, promoters may determine a continuous and fixed expression of the transgene in the transduced cell or, alternatively, promoter activity can be sensitive to certain drugs that when given to the patient can modulate promoter function, permitting regulatable expression of the transgene.<sup>6</sup> The most challenging issues for a successful application of gene therapy to treat human diseases concern the choice of the relevant therapeutic gene, of appropriate promoter and regulatory sequences and of an effective vector for delivering the transgene or transgenes into target cells. Thus gene therapy is a matter of genes, vectors, promoters, and regulatory elements. Promoter and vector features determine transduction efficacy and specificity,

duration of transgene expression, and eventually appearance of side effects.

Several approaches have been developed for transferring genes to human tissues. Plasmidic DNA can be transferred either directly, or attached to cell specific ligands, or embedded in lipidic formulations (liposomes).<sup>3</sup> On the other hand the transgene(s) can be incorporated into defective viral particles to facilitate the entry into the cells. Viral vectors are, in fact, the most efficient vehicles for gene transfer. Different viruses have served to construct gene therapy vectors, including adenoviruses,<sup>7</sup> retroviruses<sup>8</sup> (including lentivirus), AAV,<sup>9</sup> herpesvirus,<sup>10</sup> baculovirus,<sup>11</sup> SV40 virus,<sup>12</sup> vacciniavirus,<sup>13</sup> and others. The list of viral vectors is still expanding and modifications of already existing systems will increase the number of potential applications of gene therapy.

Murine retroviruses are single stranded RNA viruses, which after interaction with a retrovirus receptor can integrate in the genome of a dividing cell. Cells that do not proliferate actively under physiological conditions, such as hepatocytes, are difficult to transduce with retroviral vectors. Furthermore, transduction efficacy is limited by the low titre of virus obtained with the production procedures currently used.<sup>8</sup> The recent development of human lentiviral (human immunodeficiency virus) based vectors offers promising perspectives for gene transfer into non-dividing cells

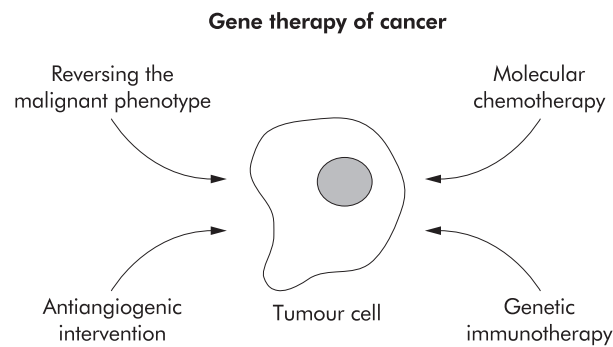
Adenoviruses are double stranded DNA viruses and serotypes 2 and 5<sup>14</sup> have been used extensively for gene therapy of cancer. Adenoviral vectors have a natural tropism for the liver, especially in rodents, do not integrate into genomic DNA, can infect very efficiently non-dividing cells and are easily produced at high titres.<sup>15</sup> Transgenes carried by adenoviral vectors are expressed transiently because of the host's immune response against viral proteins and the lack of DNA integration into the host's genome.<sup>6</sup> Recently, the so called large capacity or "gutless" adenovirus, lacking all the viral sequences except the packaging signals, have been shown to permit prolonged transgene expression.<sup>17</sup>

AAV are non-pathogenic human parvoviruses that, after deletion of all viral genes except ITR, have been used successfully as gene therapy vectors. AAV vectors do not induce significant immune response and are able to transduce

See end of article for authors' affiliations

Correspondence to: Professor J Prieto, Division of Hepatology and Gene Therapy, Department of Medicine, Clinica Universitaria, University of Navarra, Pamplona, Spain; [jprieto@unav.es](mailto:jprieto@unav.es)

**Abbreviations:** AAV, adeno-associated viruses; HSV, herpes simplex virus; GI, gastrointestinal; IL, interleukin; IFN, interferon; WHV, woodchuck hepatitis virus



**Figure 1** Different gene therapy strategies to treat cancer.

dividing and non-dividing cells. Because they can become integrated into the host's genome these vectors permit long term transgene expression.<sup>18</sup> After systemic injection AAV demonstrate significant liver tropism.<sup>19</sup> It has been shown that AAV mediated gene transfer of Factor IX to mouse liver induces persistent, curative levels of active factor IX.<sup>20</sup> AAV based vectors, as indicated for gutless adenoviruses, have considerable potential in the treatment of central nervous system disorders<sup>21</sup> and also in diseases affecting muscle,<sup>22</sup> a cell type that is readily transduced by this type of viral agents.

Herpes simplex virus (HSV) is a promising vector for gene transfer especially to the nervous system because of its characteristic neurotropism.<sup>23</sup> Additionally HSV transduces effectively murine liver tissue *in vivo*.<sup>24</sup>

## GENE THERAPY OF HEPATIC AND DIGESTIVE TUMOURS

Primary liver cancer and tumours of the pancreas and GI tract are very common neoplasms that frequently represent a medical challenge because of the lack of curative treatment when the progression of the disease precludes surgical resection. Transfer of therapeutic genes to the tumour mass or to the peritumoral tissue provides a promising new approach to treat these processes. Intense efforts are being made at both preclinical and clinical level to develop gene therapy strategies for advanced digestive tumours. Different gene therapy based approaches have been tested to treat cancer including replacement of functional tumour suppressor genes, inhibition of oncogenes, transference to tumoral cells of genes conferring sensitisation to a specific prodrug ("suicide genes"), stimulation of antitumoral immunity, and inhibition of the formation of tumoral neovessels (fig 1).

Data from pilot clinical studies have shown a limited efficacy of treatments based on suicide genes and tumour suppressor genes. Considerable hope is placed in the antitumoral effect of cytokines, such as interleukin (IL) 12, endowed with potent antitumoral activity. IL12 acts by inducing a TH1 type of response, activating NK cells and cytotoxic T lymphocytes, inhibiting tumoral neoangiogenesis, and increasing the expression of adhesion molecules on endothelial cells thus facilitating the traffic of lymphocytes to the tumour.<sup>25</sup> This cytokine, however, is toxic when administered systemically as a recombinant protein.<sup>26</sup> The rationale for IL12 gene therapy is to allow local production of the cytokine at the tumour site thus achieving high intratumour or peritumoral levels but low serum concentration, a scenario that might result in maximal antitumoral effect with minimal systemic toxicity.

In an orthotopic model of primary liver cancer in Buffalo rats we have shown that intratumoral administration of recombinant adenovirus encoding IL12 (Ad.IL12) caused complete tumour eradication in most of animals and increased long term survival. Interestingly when two tumours were separately implanted in the same liver, treatment of only

one of them resulted in regression of both.<sup>27</sup> This effect has been attributed to the fact that a proportion of the adenoviruses injected into a neoplastic nodule escapes to the general circulation and, because of their strong liver tropism, will infect the whole liver. The IL12 produced by the tumour and by hepatocytes surrounding the neoplastic nodules strongly activates NK cells, induces specific anti-tumour immunity, stimulates expression of adhesion molecules in the tumour vessels and displays a powerful anti-angiogenic effect with resulting tumour regression.<sup>27</sup> Ad.IL12 given by intra-hepatic arterial route was also shown to be efficient in the treatment of a very aggressive model of multifocal hepatocellular carcinoma in rats (induced by DENA) causing a significant reduction of tumour burden and prolongation of survival.<sup>27</sup> Ad.IL12 was also found to induce potent anti-tumour effects in animal models of colorectal cancer metastatic to the liver either by intratumoral injection or by systemic administration resulting in peritumoral gene transfer.<sup>28</sup>

Although IL12 based gene therapy demonstrates an intense anti-tumour effect it may also cause toxicity because of the ability of IL12 to induce interferon gamma production. To increase the anti-neoplastic activity of IL12 while reducing the risk of toxicity we have tested the therapeutic effect of injecting intratumorally a suboptimal dose of Ad.IL12 when given in combination with an adenovirus expressing the chemokine IP-10. The rationale was to attract immunoeffector cells to the neoplasm through IP-10 production and to activate the attracted lymphocytes with IL12. We found that this combined treatment allows reducing the dose of Ad.IL12 without losing anti-tumour efficacy but with less risk of toxicity.<sup>29</sup>

It is known that dendritic cells are the most efficient antigen presenting cells. As activation of dendritic cells is critical for the induction of anti-tumour immunity, another possible way to take advantage of the therapeutic effect of IL12 is to infect dendritic cells with Ad.IL12 *ex vivo* and to inject these engineered dendritic cells into the tumour. In animal models of colon cancer this strategy has proved to be extremely potent at eliminating neoplastic lesions and at eliciting anti-tumoral immunity.<sup>30</sup>

Stimulation of dendritic cells is widely dependent on activation by costimulatory molecules like B7 and CD40 ligand. We observed that adenovirus mediated gene transfer of CD40 ligand completely abolished the tumorigenicity of *ex vivo* infected rat hepatocellular carcinoma cells and that intratumoral injection of this adenovirus into established intrahepatic tumour nodules in rats resulted in tumour regression and prolongation of survival. Treatment of rat liver cancer with an adenovirus coding for CD40 ligand induced protective anti-tumour immunity and was devoid of significant toxicity.<sup>31</sup>

Although many of these strategies have proved very efficient antitumoral treatments in animals, there is still little information concerning the safety and efficacy of these therapeutic modalities in the different forms of malignancies in humans. Efficacy and toxicity depends greatly on the therapeutic gene, the type of the vector, the dose and route of administration, and the type of tumour being treated. Despite the fact that the total number of phase I/II clinical studies already done and presently conducted is substantial, the diversity of vectors, doses and routes of administration, and the variety of therapeutic genes used to treat different tumours make very premature the analysis of the potential of cancer gene therapy in humans. Moreover because gene therapy is still an investigational procedure many of the trials have been performed in patients with advanced tumours who have progressed despite chemotherapy (fig 2). Thus the information of the efficacy of gene therapy in early cancer in patients with intact immune system is very limited.



**Figure 2** Echo guided intratumoral injection of an adenoviral vector encoding IL12 in a patient with non-resectable hepatocellular carcinoma.

### Gene therapy of chronic viral hepatitis

Gene therapy is a promising procedure to treat chronic viral infections and to modulate chronic inflammatory processes. Chronic viral hepatitis C or B affect several hundred million people worldwide and more than 70% of patients with chronic viral hepatitis are resistant to the standard antiviral therapy with interferon (IFN)  $\alpha$ . This is an important medical problem as unresolved chronic viral hepatitis may evolve to liver cirrhosis and eventually to hepatocellular carcinoma. Recent progress has taken place with the introduction of lamivudine in the treatment of chronic hepatitis B,<sup>32</sup> with the use of combination therapy of IFN  $\alpha$  plus ribavirin in chronic hepatitis C<sup>33, 34</sup> and with the development of pegylated IFN  $\alpha$ , which generates sustained levels of IFN  $\alpha$  in the blood after one weekly injection.<sup>35</sup> However, despite these improvements a high percentage of patients with chronic viral hepatitis are still resistant to existing antiviral therapies. Gene therapy opens new avenues to treat these patients. Thus the transfer of the IFN  $\alpha$  gene to liver cells would convert these cells into an IFN factory permitting high and sustained intrahepatic levels of IFN with lower serum concentration of the cytokine thus increasing the therapeutic index of this substance. To achieve this goal it would be necessary to use hepatotropic long term expression vectors encoding IFN  $\alpha$  gene under the control of regulatable promoters responding to drugs such doxycycline or mifepristone. This therapeutic modality will make it possible to control the intrahepatic production of IFN  $\alpha$  by adjusting the oral dose of the inducer drug, thus permitting the increase of the synthesis of IFN or to stop its production according to the evolution of the viraemia. As IFN has demonstrated significant antifibrogenic and anti-tumoral activities, IFN  $\alpha$  gene therapy of chronic viral hepatitis might also prove of efficacy to prevent fibrosis progression<sup>36</sup> and the development of hepatocellular carcinoma.<sup>37</sup>

The fact that the transfer of IFN  $\alpha$  gene to the liver induces strong antiviral effects has recently been shown by using adenoviral vectors in the prevention of viral hepatitis in a mouse model.<sup>38</sup> Proof of the concept that IFN  $\alpha$  gene therapy might be an efficient and tolerable procedure to treat chronic viral hepatitis will stem from studies using appropriate long term expression vectors in animals chronically infected with hepatitis viruses such as woodchucks with chronic woodchuck hepatitis virus (WHV) infection.

Another therapeutic approach to treat viral hepatitis is the use of antisense DNA/RNA<sup>39, 40</sup> or ribozymes<sup>41</sup> to inhibit the expression of viral genes. By conveying these molecules in long term expression vectors capable of integration into the host genome, a certain proportion of hepatocytes (and their progeny) would be rendered resistant to viral infection. As

these cells might enjoy a biological advantage over infected cells, it is hoped that the replacement of dead hepatocytes might take place preferentially by the transduced cells, which finally might repopulate the entire liver. These assumptions should be tested in the future in experimental animal models.

Antiviral immunity can be stimulated by the use of genetic vaccination that is an efficient system to induce prophylactic or therapeutic immune responses. Injection of naked DNA or vectors containing viral genes, which can be combined with genes of immunostimulatory cytokines, has been shown to afford antiviral protection. In a recent work we found that gene gun bombardment of woodchucks with DNA containing the nucleocapsid of WHV together with another plasmid coding for woodchuck IL12, resulted in the generation of a strong TH1 type immune response that efficiently protected the animals against viral inoculation.<sup>42</sup> This protection was not observed when animals were vaccinated with DNA encoding WHV nucleocapsid alone. Similarly we found that combined administration of an adenovirus containing the core and E1 sequences from HCV (AdCE1) and another one containing the genes of IL12 (Ad.IL12) generated a TH1 immune response that was more intense than that obtained after the injection of Ad.CE1 alone.<sup>43</sup> Therefore, genetic vaccination offers the possibility of combining the genes coding for the antigen with genes encoding immunomodulatory cytokines thus enabling to steer the immune response in the desired direction.

### Gene transfer to GI tract and gene therapy of inflammatory bowel disease

There are a number of disorders of the intestinal epithelium that could be amenable to gene therapy. In addition, the intestine could be used as an alternative site for the production of proteins that need to be secreted to the blood for the correction of disorders such as haemophilia.

The intestinal tract has many features that make it an attractive target for therapeutic gene transfer: (a) easy accessibility via the intestinal lumen; (b) large surface area of the epithelium; (c) the possibility of *in situ* gene transfer by endoscopy; (d) known location of stem cells within the intestinal crypt, (e) intestinal cells can secrete foreign protein into the circulation.

#### Methods of gene transfer to the gastrointestinal tract

Several methods of gene transfer to the GI tract have been used. Most of the techniques target the epithelium but a sub-mucosal approach may transduce cells of the muscularis mucosae.<sup>44</sup>

It has been demonstrated *in vivo* gene transfer to various locations of the gastrointestinal tract such as oesophagus, stomach, and colon using cationic liposomes.<sup>45, 46</sup> Gene transfer was achieved by luminal instillation using catheter infusion. A high efficiency of transfection was observed in colonic epithelium, with near 100% of epithelial cells expressing the transgene. Transgene expression was transient and did not persist beyond four days, a finding that is consistent with the normal turnover time of gut epithelium. However, repeated treatments can achieve maintained expression of the foreign gene. Intramural injection of liposomes through a needle is also possible and in this case the transgene is expressed preferentially in fibroblasts.<sup>44</sup>

Genes can be transferred into the intestinal epithelium using retroviral vectors introduced intraluminally.<sup>47</sup> Given the continual proliferation of this tissue, these vectors would be an appropriate choice because of their ability to transduce dividing cells. However, transduction efficiency is comparatively low in the intestine of rat and mice and thus retroviral vectors would not satisfy the requirements of gene therapy in humans.

Adenoviral vectors have been shown to transduce intestinal cells *in vivo* when administered through an oral-duodenal



tube.<sup>48</sup> The considerable transduction efficiency of adenoviruses most probably reflects the fact that the intestine is a normal site of infection for this virus. Transfer of genetic material with adenoviruses is more successful in the small intestine as compared with the colon. Interestingly, single or repeated challenge with adenoviral vector did not cause increased host immune responses to this virus, suggesting that this type of vectors could be a good candidate for gene therapy of intestinal diseases.<sup>48</sup> Also it has been demonstrated that adenovirus mediated gene transfer to intestinal epithelial cells can effectively deliver proteins to the circulation indicating that intestinal cells have the potential to be used as heterotopic sites for production of peptidic hormones, cytokines, and proteins that should function in plasma.<sup>49</sup>

As the epithelium turns over rapidly (two to four days), the ideal targets for a stable gene transfer would be the intestinal stem cells. Permanent gene transfer is conceivable if the gene of interest can be integrated into intestinal stem cells. Accessibility of these cells to vectors delivered into the intestinal lumen is limited by their deep location and by the mucus that lines the epithelium. In vitro experiments have shown that intestinal mucus can be solubilised by a variety of agents including proteases, detergents, and sulfhydryl compounds.<sup>50</sup> In vivo pilocarpine pretreatment followed by a phosphate buffered saline flush may effectively reduce the mucus barrier in the crypts for a period of time facilitating gene transfer by either adenoviruses or other vectors.<sup>51</sup>

Another promising vector for gene transfer to the gut is the one based on AAV. It has been demonstrated that an orally administered AAV vector leads to persistent expression of a  $\beta$ -galactosidase transgene in both gut epithelial and lamina propria cells, and that this approach results in long term phenotypic recovery in an animal model of lactose intolerance.<sup>52</sup> AAV has several features that make it particularly useful for gene therapy: wild type AAV is non-pathogenic in humans, the vector lacks all viral genes minimising any possible recombination and induces only very mild immunological responses. Another singularity that makes AAV especially suitable as an orally delivered vector is its hardness being very resistant to changes in pH and temperature and to solvents. The persistent and stable gene expression for almost six months is also another interesting feature. As enterocyte turnover is three to five days, this prolonged expression indicates that progenitor cells lying within the crypts can be transduced by AAV vectors. All these features make AAV an attractive vector for GI gene transfer with potential application for vaccination purposes and for maintained protein replacement, particularly when the release of the protein into the portal circulation is a desired goal.

### Gene therapy for inflammatory bowel disease

Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases of unknown aetiology, which show a rising incidence in Western countries. Although important advances have been made in the treatment of these conditions, the process is ill controlled in a substantial number of patients with severe disease. The chronic inflammatory reaction seems to result from a pronounced activation of local mucosal type I proinflammatory immune response. IL10 plays a crucial part in the balance of the mucosal immune system, promoting physiological activation, and preventing the pathological inflammation that characterises such diseases.<sup>53</sup> Evidence from murine models suggests that IL10 induces tolerance to luminal antigen by inhibiting both proinflammatory cytokine release and antigen presentation, and also by the generation of antigen specific regulatory T cell clones.<sup>54</sup> Gene targeted IL10 deficient mice develop transmural inflammation of the small and large bowel, reminiscent of Crohn's disease. This type of inflammation is aggravated by the presence of bacteria within the gut lumen, and can be prevented by administration of

IL10.<sup>55</sup> These data lead to the consideration of IL10 as a cytokine with potential application in inflammatory bowel diseases. However, side effects have limited the use of systemically administered recombinant human IL10 (rHuIL10) in these conditions. Gene transfer of IL10 to the diseased tissues is a alternative therapeutic approach for inflammatory bowel diseases as this procedure might permit the generation of high local levels of the cytokine and concentrate its immunoregulatory activity in the bowel while avoiding systemic side effects.<sup>56</sup> At present there is an ongoing clinical trial using liposomes with IL10 DNA for local delivery to patients with inflammatory whose results are awaited with interest.<sup>57</sup>

GI directed gene therapy of inflammatory bowel disease can also use molecular constructs directed to inhibit expression of proinflammatory cytokines such as IL18 that has been shown to be highly upregulated in Crohn's disease. Recent work has shown that an adenovirus expressing IL18 antisense RNA was able to suppress IL18 by intestinal cells and to reduce IFN gamma production and the activity of experimental colitis in a murine model.<sup>58</sup>

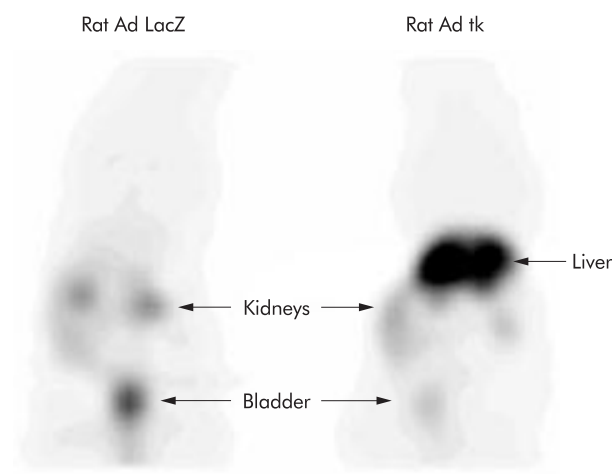
### CLINICAL IMPLEMENTATION OF GENE THERAPY

As gene therapy is in its infancy, this procedure remains an experimental therapeutic modality, which is reserved for clinical trials of serious, frequently deadly, human diseases lacking effective treatment. A great proportion of clinical studies being presently conducted concern patients with advanced untreatable tumours. These pilot studies are phase I/II trials using escalating doses of the vector to determine toxicity in the first place and secondly efficacy. Data from these trials will be essential for a real understanding of the potential of gene therapy in humans because although there is a wealth of data in rodents regarding the ability of gene therapy vectors to transduce different organs and to combat many types of malignancies, it seems possible, and even probable, that the tropism of gene therapy vectors is not the same for human and rodent tissues. Moreover, it is plausible that the antitumoral effect or the toxicity of a given vector with a specific therapeutic gene is different in rodents and in humans. Thus progress in gene therapy is basically dependent on data obtained in clinical trials.

The success or failure of a defined viral vector with a specific transgene (or transgenes) to control the growth of a particular tumour depends not only of the intrinsic biological activity of the transferred therapeutic gene but also, and very importantly, from the ability of the viral vector to infect the target tissue. To consider a particular case, although gene transfer of IL12 could be a very attractive procedure to treat metastatic colon cancer to the liver, this treatment will not succeed if the vector conveying the therapeutic gene fails to infect the tumour or the peritumoral tissue in humans. It is clear therefore that the use of appropriate molecular imaging techniques to trace the expression of the transgene are critical to analyse what are the factors determining the efficacy or lack of efficacy of a given vector to fight cancer. In other words, clinical data from pilot clinical trials of cancer gene therapy will not give all the necessary information without the knowledge of the localisation of transgene expression provided by in vivo imaging methods such as positron emission tomography<sup>59, 60</sup> (fig 3). This procedure will also be important to establish comparisons of different vectors and routes of vector administration with respect their ability to transduce a given human neoplasm. At the birth of cancer gene therapy this information is essential for the rational progress of the field.

### THE FUTURE

Gene therapy has emerged as a powerful and very plastic tool to govern biological functions of the tissues with a therapeutic aim. Animal models of human diseases and pilot clinical



**Figure 3** Positron emission tomography permits visualisation of transgene expression, when the transgene is an enzyme, by administration of a radioactive labelled substrate, as the substrate will be selectively incorporated into the tissue(s) expressing the transgene. This figure shows the positron emission tomographic image of two rats: one had received intravenous injection of  $10^{11}$  PFU of an adenovirus encoding the reporter gene LacZ (Ad.LacZ) and the other the same dose of an adenovirus encoding HSV-tk (Ad.tk). Adenoviruses have strong liver tropism and a great proportion of the injected dose transduces liver cells. Two days after vector injection animals received a dose of  $^{18}\text{F}$ HBG, a substrate of HSV-tk. It can be seen that the radioactive label accumulates in the liver only in the animal injected with Ad.tk, while in the one that received Ad.LacZ most of the radioactivity is excreted by the kidneys and accumulates in the bladder. This study shows that PET represents a useful method to trace transgene expression.

studies clearly show that there is a future for genes to be used as curative drugs. Gene therapy is at its beginning and much remains to be done before this procedure becomes of generalised use. New vectors with improved transduction efficiency, transgene capacity, toxicity profile, and duration of expression should be designed. Systems to control gene expression should be improved. Efficient therapeutic genes or combination of genes to treat those entities amenable to gene therapy should be identified. Different routes and procedures for vector administration should be tested. A regulatory policy aiming at patient and environment safety on one side and functionality of gene therapy programmes on the other should be implemented and harmonised among different countries. Innovative methods for large scale industrial production of gene therapy vectors to permit cheap dispensation of these new drugs should be developed. Finally, creative methods for the in vivo tracing of transgene expression in humans should be introduced to monitor vector distribution and transduction efficiency. In conclusion, the field of gene therapy is new but wide open. No doubt it will represent a historical step in the advancement of clinical medicine.

#### Authors' affiliations

J Prieto, M Herraiz, B Sangro, C Qian, G Mazzolini, I Melero, J Ruiz, Division of Hepatology and Gene Therapy, Department of Medicine, Clinica Universitaria, University of Navarra, Pamplona, Spain

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